Platelet function Analyzer; closure times in children with congenital cyanotic heart disease
A prospective observational pilot study

By

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DECLARATION

I, *Laura Jessica Kempe*, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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A. Abbreviations

CCHD -Congenital cyanotic heart disease

CT -Closure times

RCWMCH -Red Cross War Memorial Children's Hospital

PFA-Platelet function analyser

CADP- Collagen/adenosine diphosphate

CEPI- Collagen/epinephrine

CPB-Cardio-pulmonary bypass

RBC-Red blood cells

ACT- Activated clotting time

ABG- Arterial blood gas

VWD-Von-Willebrand's Disease

GP-Glycoprotein

TEG- Thromboelastogram

B. Protocol

B1. Introduction

B1.1 Purpose of the study

To establish the median and interquartile range or the mean and standard deviation for closure times (CT) , with the CADP and CEPI cartridges for children with Congenital Cyanotic Heart Disease) CCHD and to compare this to normal children.

B1. 2 Background

Children with CCHD are known to have haemostatic deficiencies (Zabala and Guzzetta 2015). Mechanisms to explain platelet dysfunction were postulated by Horigome et al to be as a result of the production of microparticles by the platelets (Horigome, Hiramatsu et al. 2002). It is thought that the hypoxemia causes a secondary erythrocytosis which causes an increase in red cell mass and blood viscosity which in turn increases platelet microparticles resulting in decrease platelet production, increased activation and resultant thrombocytopenia (Zabala and Guzzetta 2015). The sheer stress due to viscosity also decreases platelet counts (Zabala and Guzzetta 2015). Further postulations include suppression of the thrombomodulin-protein C and protein S pathways as a result of erythrocytosis (Horigome, Murakami et al. 2003).

Most normal paediatric coagulation parameters are highly dependent on age (Toulon, Berruyer et al. 2016). Pro-thrombin time is unchanged throughout childhood and similar to adults, whereas activated partial thromboplastin times are significantly longer in younger children (Toulon, Berruyer et al. 2016). Antithrombin, protein c and protein s are significantly higher in younger children and reach adult levels by 1-5 years of age. All clotting factors expect factor 5 are lower in neonates and reach adult levels by the end of their first year. Factor 8 and von Willebrand's are higher in younger children and reach normal values between 6-12 months of life (Toulon, Berruyer et al. 2016).

Tests for platelet function have been used for over a century, the earliest being the bleeding time, an in vivo test. The bleeding time measures the time for cessation of bleeding from a wound. It has been criticized for being non-specific, insensitive, having high inter-operator variability, and frequent scar formation (Michelson 2004, Michelson, Cattaneo et al. 2005). The gold standard test was platelet aggregometry. This test measured platelet to platelet adhesion. It has also had a lot of criticism; it has poor reproducibility, high sample volume requirement for sample preparation, length of assay time, the requirement of a skilled technician, and high expense of the test (Michelson, Cattaneo et al. 2005).

A newer test, known as the PFA, has been noted for its simplicity, rapidity and needing low sample volumes. There is no sample preparation and whole blood can be used (Michelson, Frelinger et al. 2006).The test draws up whole blood through a 150 micro-meter diameter collagen coated aperture. The time to occlude the aperture is called the closure time (CT). This is done either in the presence of adenosine diphosphate (CADP) or epinephrine (CEPI) cartridges (Michelson 2009).Citrated whole blood (0. 8 ml) is required and testing must be done within 4-5 hours of sampling. Prolonged CTs with only the CEPI cartridge are observed with mild inherited platelet function disorders (e.g. storage pool disorders) and with aspirin ingestion, while prolonged CTs with both CEPI and CADP cartridges are found with more severe inherited platelet dysfunctions (Rand, Leung et al. 2003).

Patients with CCHD often require definitive or palliative surgery for their structural problem. Often these surgical procedures require invasive surgical procedures requiring cardio-pulmonary bypass (CPB). Red blood cells (RBC) are often used to prime the CPB circuit to accommodate for haemodilution. Coagulation products such as platelets and cryoprecipitate can often be used as prophylactic coagulation cover post CPB. The PFA has been used to guide transfusions in another populations of patients requiring CPB. In patients undergoing aortic valve replacement a prolonged closure time has been observed (Sucker, Litmathe et al. 2011).This prolonged closure time correlated with increased transfusion requirements. Coagulation factors and RBC are expensive commodities. Possibly, a simple easy test such as the PFA could guide transfusion requirements in this population, costs could be reduced, and transfusion risks reduced.

B2. Methodology

B2.1 Study design

This is to be a prospective observational study. A mean and standard deviation for the population group will be established.

A comparison of PFA 100 closing times in patients with CCHD to healthy patient's PFA 100 closing times will be made.

B2.2 Characteristics of the study population (n= 100)

Inclusion criteria will be the following;

Children from birth to 16 years old diagnosed with congenital cyanotic heart disease presenting for corrective or palliative cardiothoracic surgical procedures at Red Cross War Memorial Children's Hospital (RCWMCH).

1. Invasive arterial monitoring indicated
2. Activated clotting time (ACT) monitoring and / or arterial blood gas (ABG) monitoring indicated
3. No known bleeding diathesis
4. No recent aspirin and clopidogrel ingestion
5. No anaemia or thrombocytopenia present

B2.3 Recruitment and enrollment

RCWMCH has 3 full day (Monday, Tuesday and Wednesday) and two half day (Thursday and Friday) cardiothoracic lists. Eligible patients will be identified following pre-operative admission on the day prior to surgery in the cardiothoracic ward. After reading through a patient information form (appendix 1), written and verbal informed consent will be obtained from the parent or guardian, in their own language (appendix 1) for enrolment in the study. Blood sampling for the PFA 100 will occur the following day. We expect that approximately 2-3

children per week are eligible for this study. The study will run for approximately one year, in order to recruit approximately 100 patients.

B2.4 Research procedures and data collection methods

Following routine gas inhalation induction of the child, routine placement of invasive arterial blood pressure monitors will be established. A routine ABG (1 ml), taken in a heparinized syringe, and an ACT (0, 5 ml) will be drawn.

A further 0.8 ml of whole blood will be required by the PFA for each cartridge, a total of 1.6 ml. This however will be drawn and placed in a citrated tube which requires 2.3 ml of whole blood.

The sample will be sampled within 4-5hours with the PFA 100. The intention is to move the PFA 100 analyzer to the RCWMCH, in order to eliminate the need for transport and storage of samples. A CT will be determined with both CEPI and ADPI cartridges. These results will be recorded on the data collection sheet (appendix 2). This collection will run over approximately a year.

B2.5 Data analysis

Our primary outcome will be to report the CT as a mean and standard deviation for this population or a median and interquartile range depending on data distribution.

The secondary outcome will be comparing the ranges of these patients with CCHD with the published results for healthy children, using an independent samples t-test. A p value of less than 0.05 will be considered significant. Pearson correlation with a 2 tailed test will be used for parametric data and the spearman correlation with a 2 tailed test for non-parametric data. The IBM SPSS v25 statistical software will be used.

B3. Description of risks and benefits

There are no risks involved in this study. The invasive lines and blood sampling are part of routine care for these patients during the procedure. The maximum amount of blood withdrawn at the given time will be 3,8 ml (2,3ml +0,5ml +1.0 ml). Children undergoing this type of major surgery for their pathology will be cross-matched and blood in theatre which will be used to prime the extra-corporal circulation, as well as for transfusion to the child. However, the additional amount of blood sampled for the study (2.3ml) is unlikely to result in transfusion.

B4. Informed consent process

On the day preceding the surgery written and verbal consent will be obtained from the parent or guardian of the patient in their own language. A translator will assist in the verbal process. A written consent and information form will be provided and contain information in layman terms. The guardian will be assured of the low risk for participation and allowed to withdraw consent to participate at any stage. If the guardian withdraws, they will be assured that the patient's standard of care will not be sub-standard to those participating in the study.

B5. Privacy and confidentiality

Each participant will receive a unique data collection identification number. All hard copies of data collection sheets will be kept in the Department of Anaesthesia and Perioperative Medicine in a locked cabinet of the Principal Investigator. Collated electronic data will be password protected.

B6. Reimbursement for participation

Participants will not receive any reimbursement for participation. If they consent to participation, there will be no pecuniary implications.

C. Literature review

Objectives of the literature review:

The objective of the following was to review the current literature available concerning how platelets function and the modalities available to clinicians to assess their function. The platelet function analyser was to be reviewed more extensively than the other modalities. The review also aims to compare it to the other modalities and to determine its clinical utility in common patient populations, such as obstetric and uremic patients, and clinical settings such as cardiopulmonary bypass.

Search strategy of the literature review:

Google scholar was used to obtain articles. Terms such as “platelets” , “Platelet function analyser”, “Tests of platelet function”, “Platelet dysfunction” , “Platelet kinetics” were used to obtain articles. Articles relating to the objectives of the review were used and none were excluded on any basis. The majority are published from the 2000s however older articles were reviewed in conjunction. It is important to note that most of the literature is from the adult population.

Review

- Platelet Physiology

Platelets originate from myeloid stem cells. Myeloid stem cells develop into megakaryoblasts and then megakaryocytes which then become platelets. Platelets are discoid shaped structures and are approximately 3.0 x 0.5 micrometers in dimension. The normal number of platelets is 150-450 x 10^9 per liter of blood and circulate for approximately 10 days in the vascular system. They possess a multitude of functions (Machlus, Thon et al. 2014).

Despite being commonly known for their role in primary hemostasis, they also contribute to host defense, thrombosis formation, vessel constriction, and are involved in repair and inflammation (Jenne, Urrutia et al. 2013). These blood components were discovered in the 19th century when, with real time microscopy, there was the ability to identify distinct corpuscles and see thrombi formation.

Platelets are intricate structures. They have an outer membrane which links to the open canicular system. On the outer membrane are glycoproteins (GP), which act as receptors, as well as purinergic receptors. Internally, platelets have microtubules, mitochondria and glycogen and other metabolites. They do not contain a nucleus. There are dense granules, alpha granules and lysosomal granules. The dense granules contain adeno-diphosphate (ADP)/ adeno-triphosphate (ATP), calcium and serotonin. The alpha granules contain p-selectin, platelet factor 4, platelet derived growth factor, fibronectin, von Willebrand’s factor (vWF), fibrinogen, coagulation factors 5 and 13.

A platelets role in coagulation appears complex. The coagulation cascade and the tissue-based model will not be extensively discussed here.

There are three main steps in a platelet's role in the process of primary homeostasis (Rand, Leung et al. 2003).

adhesion (Platelet to endothelium)

activation (secretion and morphology change)

aggregation (platelet to platelet)

Adhesion

On the event of vessel wall injury, collagen and the sub-endothelial layers of the vessel are exposed. The GPs on the platelet surface, in particular;

GP1b/IX/ V bind vWF and GPVI binds collagen and an integrin alpha 2, beta 1 also bind collagen. In summary; two receptors bind collagen on the endothelial surface, and one binds vWF.

This adhesive process is usually prevented by nitric oxide (NO), prostacyclin and the enzyme CD39. Prostacyclin does this by binding to a g-coupled protein receptor on the platelet and thereby increasing cyclic adenosine monophosphate (AMP) and thereby increasing the Calcium concentration within the platelets, keeping them in their "resting state. "

Activation

ADP binds to the other type of receptor (purinergic receptor) P2Y12. This activates a G inhibitory protein which reduces cyclic AMP activity and causing calcium efflux, there by activating the platelet.

Aggregation

To review the adhesive process, the combination of collagen and GPVI further activates the platelet and increases thromboxane A2 production, which further reduces prostacyclin production and results in another GP activation; the GP IIb/IIIa activation. On activation the alpha and dense granules release their substances and platelets change shape from a discoid morphology to a structure with a central body and dendritic processes.

The activated GP IIb/IIIa complex binds vWF and fibrinogen which is a rod like protein and this forms part of third component of the process, which is aggregation.

- Indications for platelet function testing

This is required for a number of reasons which are listed below (Harrison 2005);

Screening for platelet dysfunction

Screening platelet donors

Screening for non-accidental injury in minors

Monitoring desmopressin therapy

Monitoring vWF replacement in vWF disease

Monitoring pro-hemostatic therapy, when factor VIIa and platelets concentrates are used

Monitoring anti-platelet therapy

Detect drug resistance to drugs such as aspirin or clopidogrel

Detect platelet hyperfunction

Predicting surgical bleeding

Testing the quality control of platelet concentrates

- Available Tests

Several tests for platelet function have been developed, these include the bleeding time, platelet aggregometry and a number of others are also available.

The first in vivo test was called the bleeding time, and this was the most commonly used until the 1990s. With a proximal forearm blood pressure cuff inflated to 40 mmHg used, a distal surgical incision 10mm in length and 1mm in depth to the skin on the volar aspect of the forearm was made and the time to stop bleeding was recorded. Normal range was between 2 to 10 mins and severe pathology could result in bleeding times over 30 mins. This is a simple test, but evidently invasive, insensitive and time consuming (Dukc 1960).

In the 1960s the gold standard test called platelet aggregometry was developed. This uses impedance technology. Citrated whole blood is centrifuged, and platelet rich plasma is obtained. 0, 5 ml of platelet rich plasma is required at 37-degree Celsius. Agonists such as ADP, collagen, thrombin, ristocetin, Adrenalin are added. These cause platelet aggregation. As platelets aggregate light transmission through the platelet rich plasma increases.

A PFA is a relatively simple bench top instrument that simulates high shear platelet function within disposable test cartridges. Citrated whole blood is aspirated under constant negative pressure from the sample reservoir through a capillary and a microscopic aperture cut into a membrane.

The membrane is coated with either collagen/epinephrine (CEPI) or collagen/ADP (CADP). These coatings are platelet activators.

The presence of these platelet activators and the high shear rates (5000–6000m/ s ^{1}) under the standardized conditions result in platelet adhesion, activation and aggregation resulting in formation of a platelet plug within the aperture.

Platelet function is thus measured as a function of the time it takes to occlude the aperture, a CT.

The test is simple to perform, rapid with a maximal CT of 300 s and can test relatively small volumes (0.8 ml/cartridge) of citrated whole blood up to 4-5 hours from sampling. A time of over 300 s is reported as non-closure.

There are a number of other new platelet function tests which are listed below;

The cone and plate(let) analyzer test (IMPACT)

Ultegra

Hemostasis analysis system

Platelet works

Platelet flow cytometry

- Role of thromboelastography (TEG) in the evaluation of platelet function

Traditionally a TEG has been used in a trauma setting to monitor trauma induced coagulopathy (Walsh, Thomas et al. 2011). In patients that have a massive haemorrhage and goal directed blood component therapy required, a TEG can be helpful. A maximum amplitude that is decreased could indicate the need for platelet therapy (Walsh, Thomas et al. 2011).

ROTEM and TEG have been investigated as a point if care test in cyanotic heart disease (Bhardwaj, Malhotra et al. 2017). A TEG was shown to have a prolonged k time and a decreased alpha angle in this population. The EXTEM and the INTEM was deranged in 87 % and 73% patients respectively.

Interestingly in an obstetric related study, the maximum amplitude of the TEG was compared to the CT of PFA in healthy pregnant women as well as pregnant women with both mild and severe pre-eclampsia (Davies, Fernando et al. 2007). It is known that severe pre-eclampsia is known to have a greater incidence of thrombocytopenia (Sharma, Philip et al. 1999).

Often the presence of pre-eclampsia can influence the decision to perform a neuro-axial procedure or not. In the study by Davies et el, 93 pregnant women were recruited, 50 of which who had either mild or severe pre-eclampsia, the remaining 43 were used as controls. The 50 ladies with pre-eclampsia had absolute platelet counts which were lower. The maximum amplitude did not vary between the controls and the women with severe and mild pre-eclampsia. However, the CT in the pre-eclampsia women were progressively prolonged when compared to the controls.

- Clinical utility of the PFA

Currently the PFA is considered as a primary hemostatic screening and not a diagnostic tool.

In the paediatric population the PFA could be potentially used as a screening tool for both Glanzmann's Thrombocytopenia and detecting Von-Willebrand's Disease (VWD) where both the closure times for the CADP and CEPI will be grossly prolonged (Harrison 2005). Unfortunately, the closure times in screening for either hemophilia A or B in children will not be of any benefit. However it could also possibly have utility to rule out non-accidental injury when there could be a primary platelet dysfunction (Harrison 2005).

The PFA has the potential to monitor desmopressin (DDVAP) therapy for those with VWD (Favaloro 2008). The PFA has also been used in monitoring aspirin therapy and detecting resistance, The CEPI in aspirin therapy will be prolonged and the CADP will have a normal closure time (Favaloro 2008).

It is important to note that low platelet counts (< 100 X 10^9, low hematocrit (< 20 %) also may cause prolongation of closure times (Harrison, Mackie et al. 2011).

The PFA will not be able to pick up disorders of fibrinogen and coagulation factors such as VIII, IX and X1 (Favaloro 2008) as mentioned above. Vitamin K antagonists as well as heparin also have minimal effects on closure time (Favaloro 2008).

The PFA has been investigated with regards to predicting surgical bleeding, mainly in relation to cardiac surgery. It is sensitive to hemodilution, but authors believe bleeding is more related to surgical success and patient's hemostatic status which should be assessed by family and personal bleeding history pre-operatively (Favaloro 2008). There is also this potential in paediatric patients coming for cardiac surgery (Harrison 2005).

There is also further investigation needed as to whether shortened closure times are predictors of thrombosis risk, conclusions are yet to be drawn (Favaloro 2008).

- PFA vs other platelet function tests

The PFA is a fairly new test when compared with more classical platelet function tests such as a bleeding time. It has been noted that the PFA is as sensitive and reproducible in identifying the effects of aspirin when compared with a bleeding time. (Marshall, Williams et al. 1997)

Marshall et el did a double-blinded randomized control trial that set out to look at drug monitoring with the bleeding time; the long-accepted method versus the PFA.

Healthy males were randomized into receiving 750 mg of aspirin or placebo. Both the placebo group and the aspirin group had a bleeding time and a closure time performed, and it was concluded both bleeding time and closure time could identify effects of aspirin significantly when compared to the placebo group.

It is also been suggested that PFA test may be a less invasive alternative to the bleeding time in the diagnosis and therapeutic monitoring of patients with platelet secretion defects (Cattaneo, Lecchi et al. 1999).

Pregnancy

The PFA was compared to platelet aggregometry in the pregnant women, 14 who were normotensive and 16 who had pregnancy induced hypertension (Marietta, Castelli et al. 2001).

A baseline CT and platelet aggregometry was done on the blood samples from the pregnant patients. Then, L-Arginine, which is part of the nitric oxide pathway and regulates the platelet pathway, was added to repeated samples and a repeat platelet aggregometry and CT were done. The aggregometry failed to demonstrate any difference between the hypertensive and normotensive women with the addition of L-arginine, whereas the CT was significantly altered.

It was hypothesized in this study that the PFA CT can pick up subtle differences in platelet function that the traditional method could not.

Uremia and cirrhosis

Bleeding, noted in both uremia (Di Minno, Martinez et al. 1985) and liver cirrhosis (Thomas, Ream et al. 1967), is a complex multifactorial complication in these conditions and can in part be attributed to platelet dysfunction, how they interact with each other and to the vessel wall.

It is postulated that rheology of blood can affect the function of the platelets (Turitto and Baumgartner 1975) and that by improving the hematocrit the platelets can work in a more effective manner.

Platelet aggregometry and CTs were done on 20 control patients, 21 patients with end stage renal disease and 20 patients with cirrhosis (Escolar, Cases et al. 1999). In the uremic and the patients with cirrhosis the CTs were prolonged significantly when compared to the controls. The aggregometry also picked up this platelet dysfunction. However, when the hematocrit was improved, the closure times also improved however the aggregometry was unaltered.

This suggested that the PFA is a more sensitive test when hematocrit plays a role in uremic and cirrhosis related platelet dysfunction.

However, in another single centre prospective pilot study the PFA was considered as a poor predictor of skin bleeding time whereas platelet aggregometry predicted the SBT better (Ho, Gemmell et al. 2008). There were multiple limitations in this study.

PFA role in coronary artery bypass

With the use of an extra-corporal circuit such as when cardio-pulmonary bypass (CPB) is required, a coagulopathy can result (Paparella, Brister et al. 2004).

This can be a result of consumptive mechanisms, hemodilution, platelet losses as well as heparin and hypothermia, additionally contact with the foreign circuit can activate the cell-based coagulation model. Fibrinolysis and primary platelet dysfunction can also occur.

Raman et el report on 98 patients who had coronary artery bypass grafting (CABG) done, 36 patients bled post-operatively and were treated empirically with platelet therapy. 16 were

classified as platelet responders and 20 as non-responders. Platelet counts alone were unable to predict this, however closure times which were done before heparinization and 15 minutes after reversal with protamine were reported as being 94% sensitive and 85% specific for predicting which patients would have responded to platelet therapy and those who would have not. (Raman and Silverman 2001).

- Normal Ranges for closure times

A 1998 publication established closing time ranges for both CEPI and CADP cartridges for healthy children and neonates. Values reported are means ± 1 standard deviation (SD). The table below shows the results.

Table 2 : PFA-100® closure times in healthy children and neonates.(Carcao, Blanchette et al. 1998)

	n	age (years)*	Haemoglobin (g/dl) *	Platelets ($x10^9$ / l) *	CEPI *	CADP *
Heathy children	57	11-3 ±3-9 (2-5-17)	13-2 ±1-0 (11-6-15-9)	263±51 (189-401)	117±23 (83-163)	91 ± 13 (72-111)
Healthy neonates	17	>37 weeks gestational age	16-4 ±1-5^ (14-7-20-8)	277± 71 (175-466)	81 ± 17t (61-108)	56 ± 6^(48-65)

* Ranges in parentheses (minimum and maximum values). ^ Significantly different compared with children (combined data). P < 0-01.

The typical normal ranges obtained with 3.2% trisodium citrate ; 55–112 s for CADP and 79–164 s for CEPI (Harrison 2005), according to Oxford Hemophilia Centre, 2002 normal range.

D. Manuscript

D1. Abstract

Objectives: To establish the median and interquartile range or the mean and standard deviation for closure times , with the CADP and CEPI cartridges for children with CCHD and to compare this to normal children.

Design: Prospective observational pilot study

Setting: Red Cross War Memorial Children's Hospital (RCWMCH) in association with the University of Cape Town

Participants: Children between birth and 16 years old diagnosed with CCHD presenting for corrective or palliative cardiothoracic surgical procedures

Interventions: 0.8ml of whole blood obtained from the participants was pipetted into both the CEPI and CADP cartridges and analyzed by the PFA machine. Closure times for both cartridges were obtained and recorded on the data collection form.

Results: 40 successful CADP samples and 39 successful CEPI cartridges were analysed. Of the total 40 valid CADP samples there was left skewed distribution , the median was 114.50 seconds with an interquartile range from 87.25 seconds to 153.75 seconds. Of the total 39 valid CEPI samples the data was normally distributed to give a mean of 175.38 and a standard deviation of 74.998. Both of which are not significantly different from the typical normal ranges obtained with 3.2% trisodium citrate ; 55–112 s for CADP and 79–164 s for CEPI (Harrison 2005). However, when compared to the normal ranges quoted by Carcao et al for neonates and children, there was a significant prolongation for both the CEPI and CADP samples in the neonates and children with CCHD

Conclusion: This is a pilot study and limited by small sample sizes obtained due to time limitation. Further research would be needed to further assess whether the PFA could be used to guide platelet replacement in this population.

D2. Aim

The primary aim was to establish the median and interquartile range or the mean and standard deviation for closure times , with the CADP and CEPI cartridges for children with CCHD.

The secondary aim was to compare this to normal children.

D3. Methods

This was a prospective observational pilot study conducted at Red Cross War Memorial Children's Hospital (RCWMCH) in association with the University of Cape Town. Ethical approval was obtained by the human research ethics committee at the University of Cape

Town, Ref 555/2017. The data collection ran from December 2017- September 2018 over a 10-month period. The study was time limited to a 10-month period.

Children between birth and 16 years of age diagnosed with CCHD presenting for corrective or palliative cardiothoracic surgical procedures were recruited, the following were met for inclusion;

Invasive arterial monitoring indicated

Activated clotting time (ACT) monitoring and / or arterial blood gas (ABG) monitoring indicated

No known bleeding diathesis

No recent aspirin and clopidogrel ingestion

No anaemia or thrombocytopenia present

RCWMCH has 3 full day (Monday, Tuesday and Wednesday) and two half day (Thursday and Friday) cardiothoracic surgical lists. Eligible patients were identified following pre-operative admission on the day prior to surgery in the cardiothoracic ward, by the anaesthetic registrar allocated to the cardiothoracic list the following day. Written (appendix 1) and verbal consent was obtained from the parent/ guardian and an information form (appendix 2) was supplied to the parent to read. Assent was obtained from the child if over 12 years of age (appendix 3).

Following routine gas inhalation induction of the child, routine placement of an invasive arterial blood pressure monitor was established. A routine ACT (0, 5 ml) and ABG (1 ml) were drawn. A further 2,3 ml of whole blood was drawn and placed in a citrated blood collection tube. This was marked with a unique anonymous patient identification number to match the data collection form as to ensure confidentially. The collected specimen was then transported with the data collection form to Groote Schuur Hospital.

0.8ml of whole blood was pipetted into both the CEPI and CADP cartridges and analyzed by the PFA machine. Closure times for both cartridges were obtained and recorded on the data collection form.

A quality control check was run on the PFA prior to each sample being analyzed. A 5-hour interval from collection to analysis was allowed.

Data was stored anonymously in a file in the laboratory at the Department of Anaesthesia and peri-operative medicine as well as in a password protected Drobox account.

The results were analyzed with the IBM SPSS v25 statistical software. The results were reported as mean $^{+}$–SD or median (IQR)

The secondary outcome was comparing the ranges of these patients with CCHD with the published results for healthy children, using an independent samples t-test. A p value of less than 0.05 was considered significant. Pearson correlation with a 2 tailed test was used.

D4. Results

Over a 10 month period there were a total of 62 patients with CCHD presenting for corrective or palliative cardiothoracic surgery at RCWMCH. A total of 55 patients were recruited for enrolment in the study and only 49 specimens run through the PFA.

7 patients were not recruited for various reasons and 6 were recruited but not run through the PFA (Table 1).

9 of the specimens run through the CADP cartridges and 10 of the specimens run through the CEPI failed due to technical reasons with the PFA.

Of the 49 patients, cyanotic pathologies included 43 % Tetralogy of Fallot, 21 % had transposition of great vessels, 12 % had pulmonary atresia, 10 % had a double outlet right ventricle, 8 % had tricuspid atresia and 6% had other pathology (Chart 1).

Chart 1: Percentages of cyanotic pathologies of total number of specimens run (n=49)

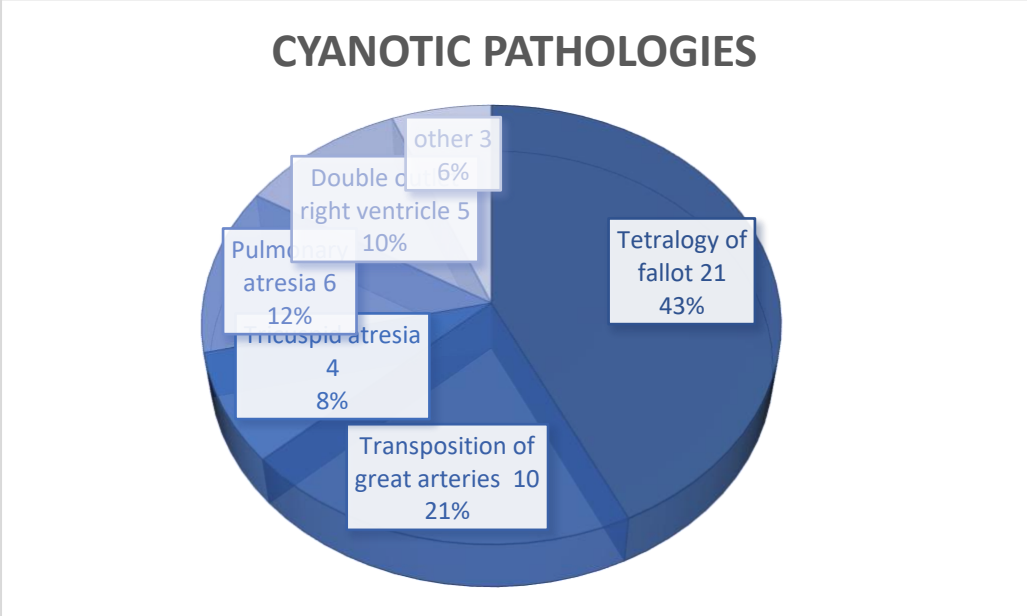

Table 1: Patients not recruited

Reason for not recruiting (n=7)	Number
Patient not identified pre-operatively	5
Parent refused consent	1
No parent available for consent	1

Recruited, but specimen not run through cartridges (n=6)	Number
Specimen placed in incorrect sample tube	1
Specimen arrived at GSH greater than 5 hours post sampling	2
Specimen forgot to be run by receiving person	1
Patient recruited but specimen not taken due to busy theatre and complicated patient	1
No one available to transport specimen	1

With regards to pre-operative haematological values such as haemoglobin, platelets and ACT , these were all normally distributed. The mean haemoglobin was 15.4 $^{+}$–2.6, the mean platelets was 359.9 $^{+}$–134.0, the mean ACT was 124.9 $^{+}$– 20.8. There was no correlation between these pre-operative haematological values and the closure times of the CADP and the CEPI samples (Table 2).

Table 2: Pre-operative Haematological values

	n	Hb (g/dl)[1]	Platelets (x10^9/l)[1]	ACT (seconds)[1]
Total	40	15.4 +-2.6	359.9+-134.0	124.9+-20.8

1.result reported as mean and standard deviation

This resulted in 40 successful CADP samples and 39 successful CEPI cartridges analysed. .

Results according to neonates

There were a total of 8 neonates (0-4 weeks) recruited. 8 successful CADP samples and 7 successful CEPI samples.

The mean and standard deviation for the CADP were 105 $^{+}$–28. When compared to the normal for this age (Carcao, Blanchette et al. 1998) The difference is significant of -49.1, 95 % CI: -63.7 -to -34.6 with a p value of <0.0001.

The mean and standard deviation for the CEPI were 179 $^{+}$– 74. When compared to normal for this age (Carcao, Blanchette et al. 1998) the difference is significant -98.6 with a 95 % CI of -137.3 to -59.9 and p value < 0.0001.

Results according to infants

There were a total of 27 infants (4weeks-4 years) with successful samples. The median value and interquartile range for the CADP sample was 115 (86 – 150)respectively. The CEPI mean was 169 $^{+}_{-}$ 74. There was no comparative data for comparison analysis in this age.

Results according to children

There were a total of 5 children age (4-16 years) successful samples with both the CADP and the CEPI.

The mean and standard deviation and standard deviation for the CADP was 170 $^{+}_{-}$72. There was a significant difference of -78.8 when compared to normal for this age, with 95% CI of -100.1 to -57.5 p value < 0.000.1.

The mean and standard deviation for the for the CEPI were 206 $^{+}_{-}$ 88. There was a significant difference of -88.8 with a CI of -119.8 to -57.8 with P value < 0.0001 (Table 3)

Table 3: Results according to age

	n	CADP(seconds)	Difference to normal for age [6] (Carcao, Blanchette et al. 1998)	CEPI (seconds)[1]	Difference to normal for age [6]
Neonates 0-4 weeks	8	105[1] +- 28	-49.130 95 % CI: -63.7 -to -34.6 p value <0.0001	179[4] +-74	-98.6 95 % CI of -137.3 to -59.9 p value < 0.0001
Infants 4weeks-4 years	27	115 [3] IQR(86 – 150)	Nil data available to compare to	169 +-74	Nil data available to compare to
Children 4-16years	5	170[1] +-72	-78.8 95% CI of -100.1 to -57.5 p value < 0.0001	206 +-88	-88.8 95% CI of -119.8 to -57.8 P value < 0.0001

1.result reported as mean and standard deviation 2. n=39 3. reported median as and interquartile range 4. n=7. 6 (Carcao, Blanchette et al. 1998)

Results for the whole cohort

Of the total 40 valid CADP samples there was left skewed distribution , the median was 114 seconds IQR(87 -154).

Of the total 39 valid CEPI samples the data was normally distributed to give a mean of 175 $^{+}_{-}$ 75 seconds.

Both of which are not significantly different from the typical normal ranges obtained with 3.2% trisodium citrate ; 55–112 s for CADP and 79–164 s for CEPI (Harrison 2005), according to Oxford Hemophilia Centre, 2002 normal range (Table 4)

Table 4: Results of entire cohort

Total	CEPI (seconds)[1]	Difference[5]	Total	CADP (seconds)[3]	Difference[5]
n=40	175[2] +-75	Nil significant	n=39	114 IQR(87 -154)	Nil significant

1.result reported as mean and standard deviation 3. reported median as and interquartile range 5.Compared to Oxford Haemophilia Centre, 2002 normal range.

D6. Discussion

The study was able to establish either the mean and standard deviation or the median and interquartile range for both the CEPI and CADP for children with CCHD. When comparing the total sample recruited in the study to the normal range for closure times, there was no significant difference. However, when the total sample recruited was divided into neonates and children both CEPI and CADP closure times were significantly prolonged when compared to the normal for age.

The research is novel, there is no prior closure time evaluation been performed on children with CCHD. Other populations known to have platelet dysfunction have been investigated, however children with CCHD however, almost exclusively, have not had their closure times performed until this pilot.

As discussed in the protocol background , it is thought that the hypoxemia causes a secondary erythrocytosis which is an increase in red cell mass and blood viscosity which in turn increases platelet microparticles resulting in decrease platelet production, increased activation and resultant thrombocytopenia (Zabala and Guzzetta 2015). It could be postulated that these abnormalities with platelet function seen in CCHD are actually able to be identified by the PFA and thereby causing prolonged closure times, as seen by the cohort in this pilot.

This prolongation of the closure times noted in this cohort echoes the similar haemostatic deficiencies that have been noted by TEG and ROTEM in previous studies (Bhardwaj, Malhotra et al. 2017).

The study is an observational pilot study so sample size is limited, mainly due to time constraints, which is the biggest limitation of the study. Unfortunately the investigators could not calculate what an adequate sample size for recruitment would be. There is limited data available regarding the normal closure times for children without pathology. The data available also only indicates normal closure times for neonates and children and excludes infants so makes a comparison in this age group not possible. In future studies possible recruitment of children without CCHD for control measurements would be of benefit.

However, even though sample sizing in the study was an obstacle, a strength of the study is that over a 10 month period there were 62 possible candidates to recruit and subsequently 40 successful CADP cartridges and 39 successful CEPI cartridges analysed, which is 64 % and 62 % respectively, which in the investigators opinion was a good total number if one was to postulate that the 62 candidates could actually be said to be a population , i.e. the amount of children with CCHD needing surgery at RCWMCH over a certain time period, and the number of successful cartridges, the sample.

It would be highly beneficial if further research could perform more closure times on healthy children so get a more reliable control for comparison and especially to establish the range of closure times for infants, as this is the largest portion of this study sample size.

If the results of this study had neglected to show any prolongation in closure time in children with CCHD, the PFA could be placed aside as an insignificant investigation in this population. Even though sample sizing is a problem, there is noted to be significant prolongation in closure times, so this follows that this investigation needs to be deemed whether clinically it has a role and whether that role is actually clinically beneficial or not. Further research would be suggested to follow this theme.

A similar study to the one by Raman et al, in which the PFA was used to predict platelet responders and platelet non-responders post CABG done on CPB bleeding, would be interesting to perform on children with CCHD who get empirically treated with platelets for bleeding peri-operatively. This would be a landmark to ascertain whether each child with CCHD should have a routine PFA done to better guide the use coagulation products.

D7. Conclusion

Congenital cardiac disease is a common presentation of congenital diseases in neonates, infants and children. Certainly the cyanotic sub-type being less common. However, the disease entity can be the cause of significant morbidity and mortality. As a discipline of anaesthetists we are aware of the risks as well as costs of the use of blood products and have seen benefit of using a guide to transfusion with tests such as TEG. Proposed guidance with a test such as a PFA in the transfusion of platelets in this population of paediatrics may seem a far off concept. However due to this observational pilot study and the noted prolongation of closure times in neonates and the children it cannot be ignored that this proposed guidance of platelet transfusion despite the evidence being in it's infancy.

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Conflict interests

Nil

E. References

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F. Appendices

F1. Consent form

Platelet function analyzer (PFA) 100 closing times in children with congenital cyanotic heart disease

An observational study: **Consent form**

Unique patient identification number:

Consent for:

Participation of child in platelet function analyzer (PFA) 100 closing times in children with congenital cyanotic heart disease: An observational study

Please initial black box or thumbprint

6. I confirm that I have read and understood the contents of the information form presented to me. I confirm that I have had an opportunity to consider the contents of the information sheet and to ask any question freely. I confirm that these questions have been answered clearly and that I am satisfied with the explanation that I have received.

2. I agree that my child's participation in this study is voluntary. I understand that I may freely withdraw my child from this study at any point prior to any medication being given in theatre, without having to give any reason for the withdrawal; and that the withdrawal will not affect my medical care.

3. I agree for my child to take part in this study and understand that approximately 3,6 ml of blood will be taken.

Name of participant	Signature/thumbprint of parent	Date
Name of person taking consent	Signature	Date
Name of witness	Signature	Date

If you have any questions about this study, please contact one of the investigators:
Dr LJ Kempe laura_kempe@yahoo.com

Dr G Wilson graeme.wilson@uct.ac.za

Questions regarding your rights as a volunteer may be addressed to the Research Ethics Committee University of Cape Town that reviewed the ethical aspects of this study at 021 406 6492. Room 52-24, Old main building, Groote Schuur Hospital, observatory.

F2. Information form

Platelet function analyzer (PFA) 100 closing times in children with congenital cyanotic heart disease

An observational study - Information form

We have noted that your child has heart disease. Only a small portion of their blood is able to reach their lungs and pick up oxygen. As a result, they sometimes look slightly blue.

Their blood is made up of three main parts; the red blood cells which carry oxygen, the white blood cells which help to fight infection. The third part are platelets. Platelets are important for forming a blood clot if there is bleeding.

Sometimes, the platelets don't work exactly as they should if a patient has heart disease.

It is possible to do a blood test called a full blood count to see how many platelets are in the blood. However, this test doesn't show how well the platelets are working. We have other tests that show us how well they are working.

There is a fairly new blood test called the platelet function analyzer (PFA 100). This test sucks up blood through a small hole and the time to close that hole with a blood clot is called a “closing time”.

The aim of this study is to see whether the closing times of patients with heart disease are different to those without heart disease.

Your child is going for a surgery and will get a type of drip put into a blood vessel to monitor the blood pressure throughout the surgery. This will be done even despite the study. From this drip, blood is going to be taken to monitor how the child is coping during the surgery. This will also be done despite the study.

We want to request that we can take an additional amount of blood (3,6 ml) at this time. The amount we need is very small and won’t affect the child.

This blood sample will then be tested on the platelet function analyzer test.

The risks are no greater than those of the routine procedure that is taking place. It is an observational study so nothing extra is being done.

You will not receive any form of payment for having your child participating in this study. Participation is completely voluntary. You may withdraw from this study at any point, without influencing your child’s treatment.

All the results of the study will be private

You are welcome to contact the study leaders with any questions at any stage;

Dr LJ Kempe laura_kempe@yahoo.com

Dr G Wilson graeme.wilson@uct.ac.za

Questions regarding your rights as a volunteer may be addressed to the Research Ethics Committee University of Cape Town that reviewed the ethical aspects of this study at 021 406 6492. Room 52-24, Old main building, Groote Schuur Hospital, observatory.

F3. Assent form

Platelet function analyzer (PFA) 100 closing times in children with congenital cyanotic heart disease

An observational study: Assent form

Unique patient identification number:

Assent for:

My own participation in platelet function analyzer (PFA) 100 closing times in children with congenital cyanotic heart disease: An observational study

I understand that I am going for an operation on my heart, as I have a heart disease that I was born with.

I understand that the doctors need to take routine blood tests when I am asleep, this is done through a type of drip in my blood vessel, that will be inserted as a routine part of my operation.

When they take these routine blood tests, I give my permission for them to take an extra 3.6 ml of blood for a test that looks at how well my platelets are working.

Platelets are small parts of the blood that help with making a blood clot if I bleed.

I understand that there are no risks involved for me, no more than routine care.

I understand that this study will not hurt me or cause me problems in any way.

I understand that I can choose not to be involved in the study at any time, without it affecting my care.

I understand that my parents also know about the study and are happy to allow me to be involved.

I understand that if I participate it may help children in the future who are having a similar operation.

I will volunteer to participate in this study.

My Name:

My Signature:

My Witness:

Date:

If you have any questions about this study, please contact one of the investigators:

Dr LJ Kempe laura_kempe@yahoo.com or Dr G Wilson graeme.wilson@uct.ac.za

Questions regarding your rights as a volunteer may be addressed to the Research Ethics Committee University of Cape Town that reviewed the ethical aspects of this study at 021 406 6492. Room 52-24, Old main building, Groote Schuur Hospital, observatory.

F4. Data collection form

Platelet function analyzer (PFA) 100 closing times in children with congenital cyanotic heart disease

An observational study: Data collection form

Unique patient identification number:

Date and time of collection:

Time of test:

Age:

Gender:

Type of cyanotic pathology:

Surgical procedure:

Pre-operative Haemoglobin:

Pre-operative platelet count:

Baseline ACT:

Closing Time; CADP: CEPI:

F5. Ethics approval letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

08 September 2017

HREC REF:555/2017

Dr G Wilson
Division of Paediatric Anaesthesia
Red Cross Children's Hospital
Rondebosch

Dear Dr Wilson

PROJECT TITLE: PLATELET FUNCTION ANALYZER (PFA) 100 CLOSING TIMES IN CHILDREN WITH CONGENITAL CYANOTIC HEART DISEASE: AN OBSERVATIONAL STUDY (MMed-candidate- Dr L Kempe)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 September 2018.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student: - Dr L Kempe will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

HREC 555/2017